

Elazaar Rabbani et al.

Serial No.: 09/439,594

Filed: November 12, 1999

Page 3 [Amendment Under 37 C.F.R. §1.115 (In Response To The  
July 30, 2003 Office Action -- August 8, 2003)]

**AMEND THE ABOVE-IDENTIFIED APPLICATION AS FOLLOWS:**

**In The Claims:**

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Claims 60-73 (Previously Elected & Previously Canceled)

Claims 74-145 (Non-Elected & Previously Canceled)

146. (Currently Amended) A process for detecting the presence of a specific target nucleic acid sequence comprising the steps of:

- 1) providing one or more first initial primers or first nucleic acid constructs comprising two segments:
  - (A) a first segment (i) being substantially complementary to a portion of said specific target nucleic acid sequence and (ii) which provides for template dependent extension: and
  - (B) a second segment being (i) substantially non-identical to said first segment, (ii) substantially identical to a portion of said specific target nucleic acid sequence, (iii) substantially complementary to sequences that are synthesized by extension of the first segment of said first initial primers or first nucleic acid constructs with said specific target nucleic acid sequences as a template and where in the absence of a denaturation step, the first initial primers or first nucleic acid constructs participate in the formation of a stem-loop

structure after said specific target nucleic acid sequence  
is used as a template for extension;

2) mixing said first initial primer or nucleic acid constructs with  
substrates, buffer, a template-dependent polymerizing enzyme and a sample  
to be tested for the presence of said specific target nucleic acid;

3) incubating said mixture under temperature conditions sufficient  
for binding of said first initial primers or first nucleic acid constructs to said  
specific target nucleic acid sequence, extension of said first initial primers or  
first nucleic acid constructs;

4) forming at least one stem-loop structure by

(a) self-annealing between said second segment of said first  
initial primer or first nucleic acid construct and a segment formed after target  
template dependent extension of the first segment of said first initial primer  
or first nucleic acid construct, and

(b) separating said first segment of said first initial primer or  
first nucleic acid construct from said specific target nucleic acid sequence,  
and

5) detecting the presence of said stem-loop structures formed in  
said forming step (4), thereby detecting the presence of said specific target  
nucleic acid sequence or its complement.

Claims 147-165 (Previously Added)

166. (Currently Amended) A process for detecting the presence of a specific target nucleic acid sequence comprising the steps of:

- 1) providing one or more first initial primers or first nucleic acid constructs comprising two segments:
  - (A) a first segment (i) being substantially complementary to a portion of said specific target nucleic acid sequence and (ii) which provides for template dependent extension; and
  - (B) a second segment being (i) substantially non-identical to said first segment, (ii) substantially identical to a portion of said specific target nucleic acid sequence, (iii) substantially complementary to sequences that are synthesized by extension of the first segment of said first initial primers or first nucleic acid constructs with said specific target nucleic acid sequences as a template and wherein the first initial primers or first nucleic acid constructs participate in the formation of a stem-loop structure after said specific target nucleic acid sequence is used as a template for extension;
- 2) mixing said first initial primer or nucleic acid constructs with substrates, buffer, a template-dependent polymerizing enzyme and a sample to be tested for the presence of said specific target nucleic acid;
- 3) incubating said mixture under temperature conditions sufficient for binding of said first initial primers or first nucleic acid constructs to said specific target nucleic acid sequence, extension of said first initial primers or first nucleic acid constructs, and wherein said extension is only carried out

using first initial primers or nucleic acid constructs comprising said two segments;

- 4) forming at least one stem-loop structure by
  - (a) self-annealing between said second segment of said first initial primer or first nucleic acid construct and a segment formed after target template dependent extension of the first segment of said first initial primer or first nucleic acid construct, and
  - (b) separating said first segment of said first initial primer or first nucleic acid construct from said specific target nucleic acid sequence, and
- 5) detecting the presence of said stem-loop structures formed in said forming step (4), thereby detecting the presence of said specific target nucleic acid sequence or its complement.

Claims 167-182 (Previously Added)

183. (Currently Amended) A process for detecting the presence of a specific target nucleic acid sequence comprising the steps of:

- 1) providing one or more first initial primers or first nucleic acid constructs comprising two segments:
  - (A) a first segment (i) being substantially complementary to a portion of said specific target nucleic acid sequence and (ii) which provides for template dependent extension: and
  - (B) a second segment being (i) substantially non-identical to said first segment, (ii) substantially identical to a

portion of said specific target nucleic acid sequence,  
(iii) substantially complementary to sequences that  
are synthesized by extension of the first segment of  
said first initial primers or first nucleic acid constructs  
with said specific target nucleic acid sequences as a  
template to form a first extended nucleic acid strand,  
and wherein the first initial primers or first nucleic acid  
constructs being participate in the formation of a stem-  
loop structure at one end of said first extended nucleic  
acid strand after said specific target nucleic acid  
sequence is used as a template for extension;

2) mixing said first initial primer or nucleic acid constructs with  
substrates, buffer, a template-dependent polymerizing enzyme and a sample  
to be tested for the presence of said specific target nucleic acid;

3) incubating said mixture under temperature conditions sufficient  
for binding of said first initial primers or first nucleic acid constructs to said  
specific target nucleic acid sequence, extension of said first initial primers or  
first nucleic acid constructs, and wherein said extension is only carried out  
using first initial primers or nucleic acid constructs comprising said two  
segments;

4) forming at least one stem-loop structure by

(a) self-annealing between said second segment of said first  
initial primer or first nucleic acid construct and a segment formed after target  
template dependent extension of the first segment of said first initial primer  
or first nucleic acid construct, and

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(b) separating said first segment of said first initial primer or first nucleic acid construct from said specific target nucleic acid sequence, and

5) detecting the presence of said stem-loop structures formed in said forming step (4), thereby detecting the presence of said specific target nucleic acid sequence or its complement.

Claims 184-200 (Previously Added)

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